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Preparation, Procedures and Evaluation of PRP Injection in the Treatment of Knee Osteoarthritis

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Corresponding Author:	Hongbo Qian Nanjing University Medical School Nanjing, Jiangsu CHINA
Corresponding Author's Institution:	Nanjing University Medical School
Corresponding Author E-Mail:	dg1535025@smail.nju.edu.cn
Order of Authors:	Ziming Chen
	Zhantao Deng
	Yuanchen Ma
	Junxing Liao
	Qingtian Li
	Mengyuan Li
	Hua Liu
	Ganghong Chen
	Chaoming Zeng
	Hongbo Qian
	Qiujian Zheng
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TITLE:

Preparation, Procedures and Evaluation of Platelet-Rich Plasma Injection in the Treatment of Knee Osteoarthritis

AUTHORS & AFFILIATIONS:

*Ziming Chen[1, 2, *], Zhantao Deng[1, *], Yuanchen Ma[1], Junxing Liao[1], Qingtian Li[1], Mengyuan Li[1], Hua Liu[1], Ganghong Chen[1], Chaoming Zeng[1], Qiujian Zheng[1]*

[1]*Department of Orthopedics, Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangzhou, Guangdong, PR China*

[2]*Shantou University Medical College, Shantou, Guangdong, PR China*

**These authors contributed equally to this work.*

Corresponding Author:

Qiujian Zheng (ZQJzqj666@yeah.net)

Email Addresses of Co-authors:

Ziming Chen (453513564@qq.com)

Zhantao Deng (dengzhantao_med@163.com)

Yuanchen Ma (myc998@qq.com)

Junxing Liao (442874370@qq.com)

Qingtian Li (398619503@qq.com)

Mengyuan Li (Leemmy@163.com)

Hua Liu (lh12181612@yeah.net)

Ganghong Chen (gh4436@163.com)

Chaoming Zeng (cmzeng92@163.com)

KEYWORDS:

Platelet-rich plasma; intra-articular injection; knee osteoarthritis; preparation of PRP; knee score scale; treatment; biotherapy.

SUMMARY:

Knee osteoarthritis is frequently seen in the orthopedic department. We introduce in detail the entire knee osteoarthritis treatment process with platelet-rich plasma injection, including preparation, procedures, and evaluation.

ABSTRACT:

Knee osteoarthritis (KOA) is one of the most frequently encountered diseases in the orthopedic department. Existing non-surgical treatments have a limited effect on the repair of cartilage and on bone regeneration. Platelet-rich plasma (PRP) is an autologous bioactive substance that can repair cartilage injury and accelerate bone regeneration effectively. However, reporting of PRP preparation protocols in clinical studies is highly inconsistent, with the majority of studies providing insufficient information to allow the protocol to be reproduced. We describe a repeatable method of preparing PRP visually, the treatment of KOA using PRP intra-articular injection, and methods of evaluating the outcome. PRP was prepared using manual double centrifugation. The PRP layer was extracted from peripheral blood and used for knee joint cavity injection. Evaluations included assessments of blood platelet concentrations and clinical outcomes. Preparation of PRP by manual centrifugation requires less apparatus and is less costly than plasma filtration or centrifugation using equipment. The centrifugation time of our double centrifugation method was 6 and 5 minutes for the respective centrifugations at forces of 800 and 1400 x g, respectively, to allow for the consistent preparation of standardized PRP. However, a manual method is susceptible to operator error, and PRP batch preparation is not available. Intra-articular injection of PRP proved to be an effective treatment for knee osteoarthritis. The entire treatment procedure took less than 30 minutes, the blood platelet concentration of PRP could be standardized, and treatment was proven to be effective when evaluated by follow-up.

INTRODUCTION:

Knee osteoarthritis (KOA) is one of the most frequently seen diseases in the orthopedic department; 30%–50% of people over the age of 65 years experience this disease[1]. At present, the conservative management of KOA mainly includes oral administration of non-steroidal anti-inflammatory drugs and cartilage nutrient drugs, intra-articular injection of sodium hyaluronate, and physiotherapy. However, these methods cannot stop the process of knee joint degeneration[2]. Articular cartilage defects can cause articular surface wear, joint instability, and metabolic changes, which are part of the pathogenesis of KOA[3]. However, because of the absence of blood vessels, nerves and lymphoid tissue in articular cartilage, recovery after damage is difficult. An effective method of repairing cartilage is especially important for the treatment of KOA. The treatment of osteoclasia is also a key focus in KOA treatment.

Platelet-rich plasma (PRP) is an autologous bioactive substance, and the application of PRP to bone and joint problems is being increasingly studied. The biological rationale for the clinical use of PRP includes its effect on the local delivery of growth factors and modification of the inflammatory response and its positive effects on cell proliferation and differentiation[4]. After activation following intra-articular injection, PRP releases α-granule through degranulation and secretes various growth factors, including the platelet-derived growth factor, the transforming growth factor-β, the insulin-like growth factor, the epidermal growth factor, the vascular

endothelial growth factor, and the fibroblast growth factor. These promote osteoblast and chondrocyte proliferation, inhibit cartilage degeneration, strengthen the stability of cartilage and subchondral bone, regulate the gene expression tissue inhibitor of metalloproteinase and maintain the balance of synthesis and degradation of proteoglycans[5,6]. Therefore, PRP can repair cartilage injury and accelerate bone regeneration.

The outcome of PRP injection is influenced by various factors, including the sampling site[7], the type of centrifuge preparation method[8], and the use of anticoagulants[9] and activators[10]. There are roughly 3 types centrifugal methods to prepare PRP. Manual centrifugation, equipment-based centrifugation, or plasma filtration techniques are available, although manual and equipment-based methods are the most commonly used. The manual method requires the least equipment, is convenient, is low cost, and is simple to perform (**Figure 1**). PRP is prepared by performance of manual centrifugation twice. Mixed peripheral blood and anticoagulant are centrifuged to separate hemocytes from plasma and blood platelets. After discarding the red blood cells on the bottom layer, the supernatant liquid is centrifuged for re-separation, dividing it into supernatant platelet-poor plasma, middle PRP, and subnatant residual red blood cells. The middle layer is used for knee joint cavity injection (if the quantity is insufficient, part of the supernatant can be drawn). Evaluations of the method include assessments of blood platelet concentration and clinical outcomes.

The reporting of PRP preparation protocols in clinical studies is highly inconsistent, and the majority of studies do not provide sufficient information to allow the protocol to be reproduced[11]. Here, we describe a reproducible method of preparing PRP and treatment of KOA with PRP intra-articular injection, with evaluation of the outcome. Inclusion criteria were patients with knee osteoarthritis who have poor pain relief for simple analgesic medication (such as acetaminophen) and conservative treatment. Exclusion criteria included patients with venous return or lymphatic drainage disorder; patients with knee joint infections; patients with a dermatosis or infection in the injection area; patients with fever; patients with coagulant function abnormality; patients with serious cardiovascular disease. The whole treatment procedure takes less than 30 minutes, and the blood platelet concentration of PRP is proven to reach a standardized measurement. Its effectiveness is demonstrated by evaluating the outcomes during close follow-up.

PROTOCOL:

The methods described were approved by the Ethics Committee of Guangdong General Hospital.

1. Obtain PRP by Manual Centrifugation

1.1. Prepare the patient in a supine position in a sterile laminar flow operating room with a comfortable room temperature and humidity: room temperature is 22 °C and room humidity is 60%.

1.2. Use a 1-mL syringe to draw 0.2 mL of heparin sodium (2 mL = 12,500 U), and then moisten a 50-mL syringe.

Note: 3 mL of sodium citrate is also typical in this step to replace the heparin sodium.

1.3. Rig a tourniquet, sterilize the elbow 2-3 times, and use the moist 50-mL syringe to draw 30 mL of peripheral blood from the elbow vein.

1.4. Perform the first centrifugation.

1.4.1. Divide peripheral blood equally into two 50 mL sterile centrifuge tubes.

1.4.2. Put two tubes in horizontal rotors and then in the centrifuge, under aseptic conditions.

1.4.3. Centrifuge for 6 minutes at 800 x g.

1.4.4. Take the horizontal rotors out, wear sterile gloves, and take centrifuge tubes out.

1.4.5. Observe the stratifications to make sure that the peripheral blood is stratified into two layers.

1.4.6. Use a clean 10-mL syringe to collect the supernatant liquid from these two centrifuge tubes into a clean centrifuge tube.

1.5. Perform the second centrifugation.

1.5.1. Use a clean 10-mL syringe to add an equivalent amount of sterile water or normal saline into another clean centrifuge tube for balance. Put the tubes in the horizontal rotors. Mark the one with the supernatant layer liquid by adhesive plaster.

1.5.2. Centrifuge for 5 minutes at 1400 x g.

1.5.3. Take the horizontal rotors out, and by observing the stratifications check that the liquid of the marked tube is divided into three layers.

1.5.4. Use a 10-mL syringe to draw 4 mL of liquid from the middle granular cell layer (leukocyte-rich, PRP layer) and the bottom layer of supernatant. If the middle layer quantity is sufficient, just draw 4 mL from that.

1.5.5. Put 0.4 mL of the liquid in a sterile anticoagulation tube (K_2EDTA, 3.6 mg) for evaluation, leaving 3.6 mL of liquid remaining in the syringe.

Note: The protocol can be paused here.

2. Intra-Articular Administration of PRP

2.1. Let the patient lie supine on the operating table and bend the knee to 90 degrees.

2.2. Locate the puncture site at the inferior margin of the patella and 1 cm from the lateral patellar ligament. Use a marker pen to mark the site.

2.3. Perform skin sterilization on the puncture site three times with anerdian III, wearing sterile gloves, and cover with an aseptic hole-towel.

2.4. Place the syringe parallel to the tibial plateau, and then perform the puncture at an angle of 45 degrees. Completely insert the needle into the skin.

2.5. Inject the 3.6 mL of PRP from the syringe into the knee joint cavity.

2.6. Cover the puncture position with sterile gauze and fix it with adhesive plaster.

2.7. Apply pressure to the wound for 10 minutes. Observe for any severe adverse reaction for 30 minutes.

2.8. Administer a total of three injections at monthly intervals.

Note: The protocol can be paused here.

3. Postoperative Evaluation of PRP Injection

3.1. Evaluate the concentration of blood platelets in the PRP.

3.1.1. Use the 0.4 mL of PRP from the anticoagulation tube (K_2EDTA, 3.6 mg).

3.1.2. Analyze the blood platelet concentration of the PRP using an automatic blood cell analyzer.

3.2. Evaluate the postoperative outcome of the PRP injection.

3.2.1. With a consultation 1 day before each of the three treatments, conduct further patient follow-up 1 day after each treatment, 3 days after each treatment, 1 week after each treatment, 1 month after the third treatment, 3 months after the third treatment, and 6 months after the third treatment.

3.2.2. Use a visual analog scale (VAS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Knee Society Score (KSS), and Lysholm knee functional scale to evaluate the postoperative effect.

REPRESENTATIVE RESULTS:

As a result, the platelet count of the PRP reached a standard concentration level of 1121 x 10^3/μL. We conducted the 15 follow-up surveys described in the protocol on a 55-year-old male patient with early KOA. It was obvious that early clinical outcome was satisfactory after the intra-articular administration of PRP (**Figure 2**). However, medium-term efficacy was slightly inferior. A markedly significant analgesic effect was observed (**Figure 2A**). KSS knee score was higher than KSS function score (**Figure 2C**, **Figure 2D**), which meant the effect of PRP on subjective symptoms was better than on objective symptoms. The Lysholm knee functional scale scores indicated that our method had an evidential effect in improving cartilage injury and soft-tissue injury symptoms (**Figure 2E**). Overall, the therapeutic effects of our PRP protocol were notable.

FIGURE AND TABLE LEGENDS:

Figure 1: Flow chart of the protocol design.

Figure 2: Evaluation of postoperative outcome of PRP injection. Evaluation of clinical outcome by VAS (**A**), WOMAC (**B**), KSS (**C**, **D**), and Lysholm knee functional scale (**E**). KSS provides knee score (pain, mobility, and stability, **C**) and function score (walking ability and stair activity**, D**).

DISCUSSION:

The concentration of blood platelets in normal human blood is between 150,000/μL and 350,000/μL, and it is widely believed that blood platelet concentration of PRP should reach 1,000,000/μL, which is 3 to 5 times normal concentrations[9]. According to the PAW hierarchy system, it is believed that PRP has no obvious effect when the blood platelet concentration is

less than three times the normal concentration, while PRP has an inhibiting effect when its blood platelet concentration is more than six times the normal concentration[12]. Therefore, a minimum requirement for this protocol is that the blood platelet concentration of PRP is between these levels.

Manual PRP preparation can be achieved by a single centrifugation or with two rounds of centrifugation. Due to the different centrifuge parameters, the quality of the PRP obtained differs between the two techniques. The blood platelet concentrations obtained by single centrifugation are low, but the PRP does not contain both white and red blood cells. The blood platelet concentrations obtained by double centrifugation are high, and the PRP usually contains a small amount of white blood cells and even red blood cells[13]. Whether the existence of white blood cells in PRP is beneficial to outcomes is disputed. Some studies have shown that white blood cell-rich PRP has stronger antimicrobial activity, facilitates functional recovery, and is less of an irritant, reducing the need for analgesics[14]. However, cytokines, metalloproteases, interleukins, and oxygen free radicals released from white blood cells can aggravate damage in the acute stage, obstructing the self-repair of tissues and delaying the healing process[15]. To obtain blood platelets in higher concentrations, we used double centrifugation.

The collection rate of blood platelets is also related to centrifugal force and time. It is generally acknowledged that blood platelet concentration increases as centrifugal force increases. However, excessive centrifugal force will damage blood platelets, reducing the recovery rate. Platelet concentration also increases with length of centrifugation. When centrifugation is less than 5 minutes, blood platelets are low, and no significant effect will be obtained; between 10 and 20 minutes of centrifugation, the blood platelet concentration gradually and steadily increases; after more than 20 minutes, the blood platelet concentration no longer obviously changes[16]. A lengthy centrifugation may cause excessive platelet deposition and reduce bioactivity, so the optimal centrifugation time is between 5 and 10 minutes. We established an optimal centrifugation time for double centrifugation of 6 and 5 minutes for the first and second centrifuge at forces of 800 and 1400 x g, respectively, to prepare the PRP.

Some PRP is harvested and directly injected into the area of injury, but other formulations add a platelet-activating agent such as thrombin or $CaCl_2$. In general, PRP used to relieve chronic inflammation or “wear and tear” injuries such as OA is usually injected without an activating agent[17].

For the evaluation of treatment outcomes, we used a number of scales. The VAS provided detailed pain measurement. We used the WOMAC to evaluate the severity of gonitis and the treatment effect according to relevant symptoms and signs. The KSS provided a knee score for

pain, mobility, and stability and a function score for walking ability and stair activity. The Lysholm knee functional scale evaluated ligament and cartilage injury.

There were several limitations to our method. First, manual preparation is susceptible to operator error, mainly from subjectivity in the drawing of the middle buffering layer and part of the supernatant liquid after the second centrifugation. However, after repeated experiments with different operators, we finally found the effective platelet concentration. Second, PRP batch preparation is not available using this method. PRP can only be prepared by drawing blood before each injection. As storage of samples is difficult, it must be acceptable to re-prepare PRP every time.

In summary, intra-articular injection of PRP is an effective treatment for knee osteoarthritis. This study report provides the whole treatment procedure in detail, including the preparation of PRP of reliable quality, the introduction of a standard injection procedure, and a scientific and practical evaluation plan.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

Figure 2

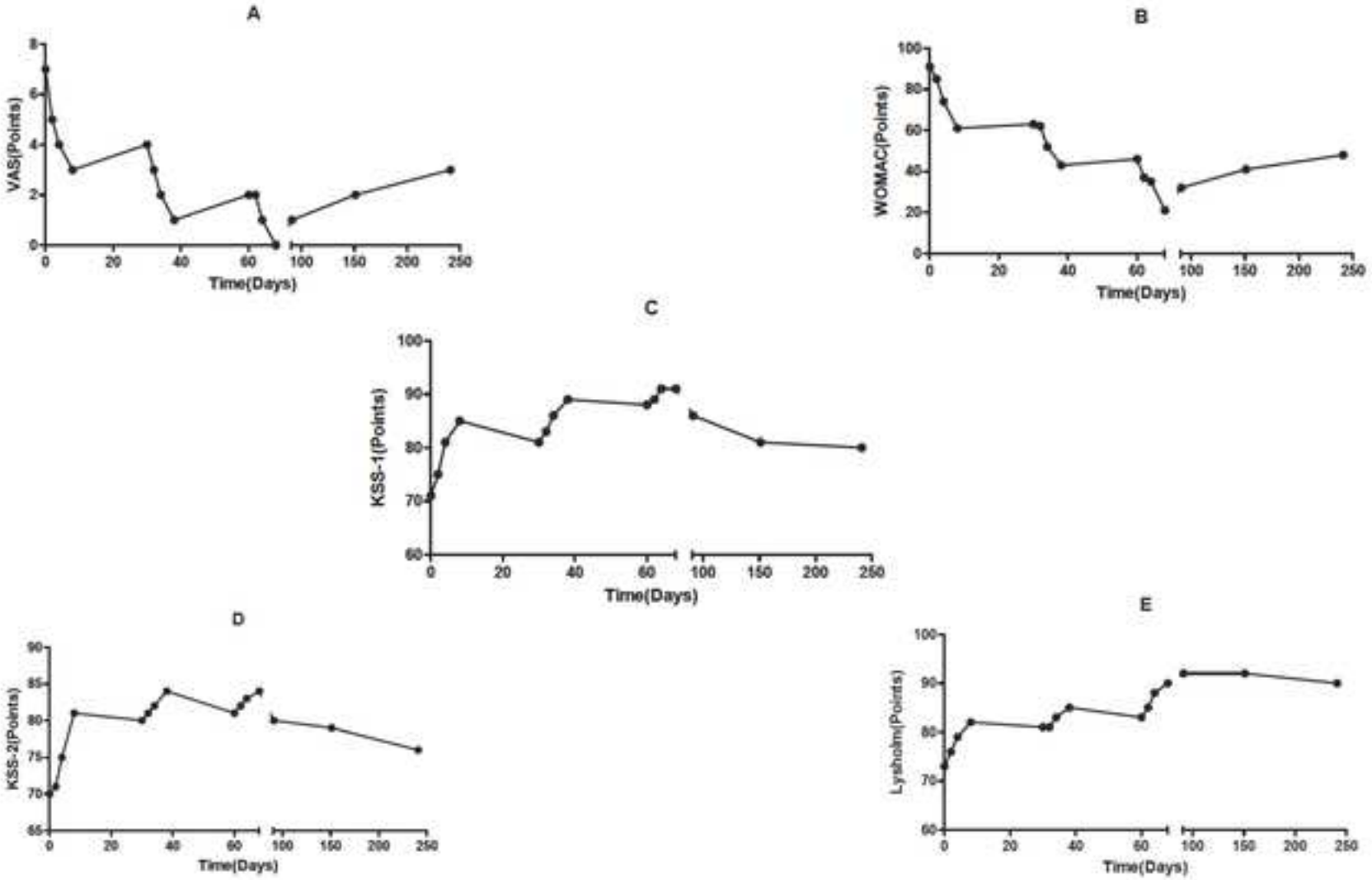

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials PRP.xls

Name of Material/ Equipment
Centrifuge
Centrifuge tube
Horizontal rotor
Anerdian Ⅲ
1ml Syringe
10ml Syringe
50ml Syringe
Medical Tourniquet
Single-use sterile rubber surgical gloves
Disposable Draw Blood Needle
Heparin Sodium Injection
Jifro Hand Antiseptic Rinse Free Gel
Medical Cotton Swab
10ml Normal Saline
Automatic Blood Cell Analyzer
Hole-towe
Anticoagulation Tube(Blood Collection Tubes, K2E 3.6mg)
Tweezers
Sterile Gauze
Adhesive Plaster
Skin Marker Pen

Company	Catalog Number
Eppendorf	5702
CORNING	430828
Eppendorf	LL080
Shanghai Likang Disinfectant HiTech Co., LTD	310173
KDL Medical Equipment Co., LTD	0.4*13 RWLB
KDL Medical Equipment Co., LTD	1.2*38 TWSB
KDL Medical Equipment Co., LTD	0.7*32 TWLB
Changzhou Jinli Latex Products Co., LTD	0087-2011
Shanghai jinxiang Latex Products Co., LTD	17060
KDL Medical Equipment Co., LTD	0.55*20 L(Ⅱ) RWLB
SPH No.1 Biochemical & Pharmaceutical Co., LTD	1706101
Shanghai Likang Disinfectant HiTech Co., LTD	311793
Foshan Kangzheng Medical Supplies Co., LTD	KZ3-12
Jiangxi Shuangshi Pharmecutical Co., LTD	140211458
Beckman Coulter	LH-750
Becton, Dickinson and Company	CNL17-COO56
RWD LIFE SCIENCE	F12006-10
Guangdong Ze Chang Trade Co., LTD	170915
3M Transpore	1527C-0
Guangzhou Mingjia Medical Device Manufacturing Co., LTD	10110

Comments/Description

Disinfect the skin

2ml:12500U

Disinfect the skin

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CORRESPONDING AUTHOR:

Name:	Qiujian Zheng
Department:	Department of Orthopedics
Institution:	Guangdong General Hospital (Guangdong Academy of Medical Sciences)
Article Title:	Preparation, Procedures and Evaluation of PRP Injection in the Treatment of Knee Osteoarthritis
Signature:	
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Dear Dr. Nguyen,

Thank you very much for your letter and advice on our manuscript Preparation, Procedures and Evaluation of Platelet-Rich Plasma Injection in the Treatment of Knee Osteoarthritis. In the current form, we have modified our manuscript according to the editorial and peer review comments.

Editorial and production comments:

Changes to be made by the Author(s) regarding the written manuscript:

Advice 1: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Answer 1: Thanks very much for the comment. As suggested, we have checked the manuscript again to ensure that there are no spelling or grammar issues.

Advice 2: For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

Answer 2: Thanks very much for the comment. As suggested, we have changed the in-text formatting.

Advice 3: JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Corning, Eppendorf, etc.

Answer 3: Thanks very much for the comment. As suggested, we have removed all commercial language from the manuscript.

Advice 4: Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Answer 4: Thanks very much for the comment. The changes are marked in red

color throughout the revised manuscript.

Advice 5: What are the inclusion/exclusion criteria of the patients?

Answer 5： Thanks very much for the comment. The inclusion/exclusion criteria of the patients have been added in the revised manuscript, marked in gray color shadow.
Inclusion criteria: patients with knee osteoarthritis who have poor pain relief for simple analgesic medication (such as acetaminophen) and conservative treatment.
Exclusion criteria: patients with venous return or lymphatic drainage disorder; patients with knee joint infections; patients with a dermatosis or infection in the injection area; patients with fever; patients with coagulant function abnormality; patients with serious cardiovascular disease.

Advice 6: Please do not abbreviate journal titles.

Answer 6: Thanks very much for the comment. The changes are marked in orange color throughout the revised manuscript.

Changes to be made by the Author(s) regarding the video:

Advice 1: Please increase the homogeneity between the video and the written protocol.

Answer 1: Thanks very much for the comment. In current version, we have made the written protocol more detailed and increased the homogeneity between the video and the written protocol, marked in red color throughout the revised manuscript.

Advice 2: Please stabilize the video images whenever possible. The camera work is very shaky throughout.

Answer 2: Thanks very much for the comment. We feel very sorry that we did not stabilize the video images in the previous edition. We have executed anti-shake processing and re-recorded the very shaky part of the video. We think it can clearly convey the content of the protocol in current edition. However, please feel free to tell us and we will re-record the whole video if you think it is necessary.

Advice 3: Are gloves required throughout? There is no glove usage during the preparation of the heparin syringe.

Answer 3: Thanks very much for the detailed comment. We agree that glove can protect the operator. However, drawing medicine from an Ampule is a very common

operation and it is normally considered that gloves are not necessary to draw medicine in China.
Sterile gloves are required during the preparation of the centrifugation and the injection and we have mentioned in the protocol.

Advice 4: There are some words that are still mispronounced: centrifugation, JoVE (one-syllable), etc.

Answer 4: Thanks very much for the detailed comment. We have invited a native English speaker from New York to record the audio. The reason why there are still some mispronunciations is he is not familiar with the medical terminology. Anyway, we have revised the mispronunciations in the current edition.

Advice 5: 1:50 - "The" should be removed from this text overlay.

Answer 5: I feel very sorry about this fault. We have corrected it in the current edition and check the video to make sure there is no other similar mistake like this.

Advice 6: The black text against this background is a bit difficult to read. We recommend either lightening the background, or making the text white and adding a black drop shadow.

Answer 6: As suggested, we added a white shadow to lighten the background.

Advice 7: It appears that it is an actual patient being demonstrated upon in the video. We do see the patient's face in some of the shots. Does the patient's face need to be obscured for privacy reasons?

Answer 7: Thanks very much for the detailed comment. Actually this patient is also an author (Hua Liu) of this article. And he is very willing to act in the video. Thanks for protecting patient’s privacy again.

Reviewers' comments:

Advice 1: Please, in the video, improve the sound quality of narration voice

Answer 1: I feel very sorry about some unclear voice. We have improved the sound quality especially in Conclusion part in the current edition.

Advice 2: Remove commercial image of PRP kit, because a commercial kit it is not used.

Answer 2: Thanks very much for the comment. As suggested, we have removed the commercial image of PRP kit.

Advice 3: Please improve the quality of graphics of the "Point V" - Representative results.

Answer 3: Thanks very much for the comment. As suggested, we have improved the quality of graphics of the "Point V" - Representative results.

In Abstract：

Advice 1: "osteanaphysis" is a correct term, but it is not usual. Please replace by " bone regeneration" or similar.

Answer 1: Thanks very much for the comment. The changes are marked in yellow color shadow throughout the revised manuscript.

Advice 2：Please clarify the meaning of "The centrifuge method in preparation included 3 types"

Answer 2：I feel very sorry about this. Actually, what we mean is that there are roughly 3 types centrifugal methods to prepare PRP, including manual centrifugation, equipment-based centrifugation, or plasma filtration techniques. The changes are marked in cyan color shadow in Introduction part.

In protocol：

Advice 1: The anticoagulant used is heparin sodium. Why the authors choose this type of anticoagulant? It is not usual in PRP technology, in contrast to sodium citrate, or ACDA.

Answer 1: Thanks very much for this important comment. Sodium citrate is also usual to be used to replace heparin sodium in my hospital, and we have added this information in the revised manuscript, marked in green color. However, heparin sodium is also efficient for impedance aggregometry in PRP preparation, according to some research and our experience [1] [2]. Actually we are researching about the anticoagulants selection in PRP preparation in another study.

Advice 2: In 1.5.4. please change "karyocyte" to leukocyte.

Answer 2: We agree with the reviewer and the change are marked in blue colour.

Advice 3: The PRP is activated with thrombin and/or CaCl2?

Answer 3: The PRP is not activated with any activating-agents in this protocol. According to some articles, some PRP is harvested and directly injected into the area of injury. In general, PRP used to relieve chronic inflammation or "wear and tear" injuries is usually injected without an activating agent[3]. Thanks very much for the comment. The changes are marked in red color shadow in Discussion part.

In Reference:

Advice: Please unify the references style. For example, name and surname in references #3 and #5 and no in #1 and #2

Answer: Thanks very much for the comment. As suggested, we have unified the references style.

In Figures:

Advice: Figure 1: Please remove the commercial kit for PRP collection (the same in video), because the authors use a noncommercial system for obtaining PRP.

Answer: Thanks very much for the comment. As suggested, we have removed the commercial kit for PRP collection in Figures and in video.

We hope that the revision is acceptable and look forward to hearing from you soon.

With best wishes,
Ziming Chen

Reference：

1 Solomon, C. *et al.* Influence of the sample anticoagulant on the measurements of impedance aggregometry in cardiac surgery. *Medical Devices (Auckland, N.Z.).* **1** 23-30 (2008).

2 Zhou, S. F. *et al.* Autologous platelet-rich plasma reduces transfusions during ascending aortic arch repair: a prospective, randomized, controlled

trial. *The Annals of Thoracic Surgery.* **99** (4), 1282-1290, doi:10.1016/j.athoracsur.2014.11.007, (2015).

3 Cohn, C. S. & Lockhart, E. Autologous platelet-rich plasma: evidence for clinical use. *Current Opinion in Hematology.* **22** (6), 527-532, doi:10.1097/MOH.0000000000000183, (2015).